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Growth Response and Antibacterial Activity Test of Sintrong Plant (*Crassocephalum crepidioides* (Benth.) S. Moore) on different Growth Media and Shade

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This study investigated the growth response and antibacterial activity of sintrong plants (*Crassocephalum crepidioides* (Benth.) S. Moore) on different growth media and shade to support organic functional food. The research was conducted at the Field Laboratory of the Faculty of Agriculture, Halu Oleo University using a two-factorial Randomized Group Design (RAK). Variations of planting media used were soil without fertilizer, a mixture of soil and cow manure in a ratio of 1:1, and a mixture of soil and cow manure in a ratio of 1:2. The variations of shade used were no shade, 25% paranet shade, and 50% paranet shade. Antibacterial activity was assessed using the Delusion method. Growth data and antibacterial activity were analyzed through Analysis *of Variance* (ANOVA) with SPSS application and continued with Duncan test at 95% confidence level ($\alpha = 0.05$). The results showed that planting media and shade had a significant effect on the growth of sintrong plant height. The greatest growth occurred in a mixture of soil and manure 1:1 in 25% shade (P_1N_1), while the lowest growth occurred in a mixture of manure-soil 1:2 without shade (P_2N_0). Antibacterial activity testing showed a significant effect of various growing media and shade treatments. The highest antibacterial activity was found in sintrong plant extract grown without manure at 50% shade (P_0N_2), with an inhibition zone of 16.6 mm for *Escherichia coli* bacteria (gram negative) and 13.2 mm for *Staphylococcus* bacteria (gram positive). The lowest inhibition zone occurred in sintrong plants grown on 1:2 manure-soil mixture without shade (P_2N_0) with an inhibition zone of 8.0 mm for *Staphylococcus aureus* bacteria.
Keywords: Growth Response, Antibacterial Activity, Sintrong Plant (*Crassocephalum crepidioides* (Benth.) S. Moore), Media, Shade.

INTRODUCTION

Indonesia is known to be rich in plants with medicinal properties, both those that grow wild and those that are deliberately planted, so it is easy for people to get these plants. In utilizing these medicinal plants, people use them based on hereditary experiences that they still believe in today (Wahidah *et al.,* 2021). Medicinal plants are very useful in meeting human needs (Jamshidi-Kia *et al.,* 2018). In the pharmaceutical world, medicinal plants are a source of raw materials for traditional and modern medicines. Currently, there is a tendency for people to consume traditional medicines, due to changes in lifestyle back to nature and the high cost of modern medicines which make the demand for medicinal plants higher, not only in Indonesia but also in the world (Shakya, 2016). The prospect of medicinal plant cultivation cannot be separated from the development of the herbal medicine industry both at home and abroad due to the back to nature lifestyle (Sofowora *et al.,* 2013). As well as the increasingly widespread use of medicinal plants for other industrial purposes outside the traditional medicine and pharmaceutical industries. The cultivation of medicinal plants in Indonesia still faces many obstacles in terms of production, including the implementation of unprofessional medicinal plant cultivation activities, Astutik *et al.* (2019) the inability of farmers to maintain the quality of medicinal plants and the lack of attention of the medicinal plant industry to the results of scientific research in product development efforts (Adi *et al.,* 2022). One of the medicinal plants known to the public is

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the sintrong plant (*Crassocephalum crepidioides* (Benth.) S. Moore).

Sintrong plant (*Crassocephalum crepidioides* (Benth.) S. Moore). is known as a weed plant, and no one has grown it as a native vegetable crop (Silalahi, 2021). Sintrong plant (*Crassocephalum crepidioides* (Benth.) S. Moore) in some areas has been used its leaves in addition to fresh vegetables, it is also useful for treating various types of diseases. to heal wounds, treat stomach pain and improve blood circulation (Can & Thao, 2020). Sintrong leaves are also traditionally used as nutraceuticals and are also believed to treat various diseases, such as indigestion, headache, anthelmentic, anti-inflammatory, antidiabetic, and antimalarial (Adjatin *et al.,* 2013). Sintrong plants (*Crassocephalum crepidioides* Benth. S. Moore) contain chemical compounds such as saponins, flavonoids and polyphenols that have medicinal properties. The content of sintrong plant leaves also contains vitamins A, D, and K (Gurung *et al.,* 2022).

The purpose of this study was to assess the growth response and antibacterial value of sintrong plant extract (*Crassocephalum crepidioides* (Benth.) S. Moore) on different growing media and shade in supporting organic functional food.

MATERIALS AND METHODS

***Materials and tools*:** The tools used in this study included two parts. The first part for growth includes the use of paranet (artificial shade) 25%, paranet (artificial shade) 50%, 25x20 cm polybags, hoes, and sacks. The second part for antibacterial activity test includes Petri dish, Erlenmeyer, beaker, measuring cup, analytical balance, rotary evaporator, vacuum pump volume pipette, dropper pipette, tube rack, test tube, micro pipette, hot plate, stirring rod, magnetic stirrer, freezer, separating funnel, funnel, scissors, digital scale, water bath, spatula, digital camera, stationery, shaker, UV-visible spectrophotometer, reagent bottle, incubator, and measuring flask.

The materials used were sintrong plant (Crassocephalu crepidioides), cow manure, methanol, distilled water, NA media, tetracycline 1000 ppm, distilled water, and sintrong plant extract.

***Working Methods*:** The growth aspect began with sowing to obtain seeds within 3 weeks on a mixture of husk, soil and manure, then transferred to 25 x 20 cm polybags, each of which had been filled with 3 variations of planting media (no manure, soil and manure mixture 1:1, soil and manure mixture 1:2), each planting media was then kept in 3 conditions, namely no shade, 25% shade, and 50% shade. Harvesting was done at the age of 60 days after planting (HST). Then the height of plant growth was measured for all types of treatment.

***Aspects of antibacterial value determined by the dilution method. Working Procedure for Antibacterial Power Test Media Production*:**

1. Production of NA (Nutrient Agra) Media: NA (Nutrient Agar) media is a solid bacterial growth medium used for rejuvenation and antibacterial testing. The NA media used was synthetic NA media (Himedia®) made with a composition of 28 grams in 1 L of distilled water. After that, the media was heated using a hot plate and homogenized using a magnetic stirrer.
2. Preparation of Semi-solid NA Media: Semi-solid NA medium is a bacterial growth medium used for antibacterial testing. This medium was prepared using NB (Nutrient Broth) (Merck®) which was added with agar with a composition of 8 grams of NB and 9 grams of agar for every 1 L of distilled water. After that, the media was heated using a hot plate and homogenized using a magnetic stirrer.

***Sterilization of Tools and Materials*:** Tools and materials to be used were sterilized first. Sterilization of tools and media was carried out using an autoclave at 121o C with a pressure of 15 psi for 20-30 minutes for tools and 15 minutes for media (Lay, 1994).

***Rejuvenation of Test Bacteria*:** Rejuvenation of test bacteria was done by inoculating bacterial isolates of E. coli ATCC 35218 and S. aureus ATCC 25923 into the slanted NA medium by scratch method, then incubated at 37°C for 24 hours.

***Preparation of Antibiotic Solution*:** Antibiotics are compounds used as positive controls in antibacterial testing. Tetracycline was used at a concentration of 1000 ppm.

***Preparation of Mc. Farlands 0.5*:** **Mc**. Farland 0.5 is a solution used to estimate the number of bacteria based on their turbidity. If the bacterial suspension has the same turbidity as the Mc. Farland 0.5 standard, it can be assumed that the number of bacteria contained in the suspension is 1.5-2 x 108 cfu/mL.

***Preparation of Test Bacterial Suspension*:** Test bacterial suspensions were prepared by inoculating the rejuvenated bacterial isolates into 0.9% NaCL solution, then vortexed until homogeneous. After that, the turbidity was compared with Mc. Farland standard 0.5 to determine the number of bacteria in the suspension. If the suspension had the same turbidity, the suspension was put into semi-solid NA media in a ratio of 1:10 (bacterial suspension:semi-solid NA media).

***Antibacterial Power Test*:** The antibacterial power test was carried out by first pouring NA media into a Petri dish as a base layer. After the media solidified, the buffer was aseptically inserted on the surface of the media and then poured half-solid NA media containing the tested bacterial suspension into the Petri dish and waited until it solidified. After that, the reserve is taken aseptically and carefully so that the holes formed are not damaged. A total of 100 μL of test compounds (including K (+) and K (-)) was put into the wells

aseptically and incubated at 37°C for 24 hours. The diameter of the clear zone formed was measured vertically, horizontally and diagonally using a caliper to obtain the antibacterial activity with the following equation.

RESULTS AND DISCUSSION

***Plant Height Growth*:** The results of ANOVA analysis showed that the treatment of planting media and shade had a significant effect on the growth of sintrong plant height (*Crassocephalum crepidioides* (Benth)). S. Moore). The highest growth was seen in planting on mixed media of soil and manure in a ratio of 1:1 in 25% shade (P_1N_1). The provision of manure is an effort to fulfill the nutrients needed for the growth and development of Sintrong *Crassocephalium crepidiodes* (Benth) S. maximum.

Gurmu (2019) states that organic matter has an important role in soil because it helps retain water, so that water availability is better maintained, increases cation exchange capacity or nutrient availability, especially N, P, and K. Wibowo & Kasno (2021) Organic matter improves soil aeration and root system development, and stimulates the growth of soil microorganisms which greatly help the process of decomposition of soil organic matter.

The results of laboratory analysis show that the N content is 0.459 mg, this value is in the sufficient category for growth, the form of N in the manure used in this study is available to plants because the manure used is mature and has gone through fermentation. Yao *et al.* (2020) The element N for plants is needed in the synthesis of amino acids and proteins used in the formation of plant body structures. Sara *et al.* (2019) Cow dung fertilizer is better than other natural fertilizers and artificial fertilizers, because it is humus which contains organic compounds and is a source of macro nutrients that are important for plant growth and development. N nutrients in fertilizers can stimulate plants to form amino acids into proteins (Torres *et al.*, 2023). The proteins formed are used to form growth hormones, namely auxin, gibberellin, and cytokinin hormones. Gibberellins will increase metabolic activity and photosynthetic rate (Wu *et al.*, 2021).

The results showed that sintrong plant (*Crassocephalium crepidiodes* (Benth) S. Moore). is a plant that is tolerant to shade, both at 25% shade and 50% shade where the growth of plant height looks higher than plants that are maintained without shade. The height of sintrong plants (*Crassocephalum crepidioides* (Benth) S. Moore) is a morphological adaptation. Plants to maximize the acquisition of sunlight energy that will be needed in photosynthesis. Solbach *et al.* (2021) Sunlight energy also plays a role in the transfer of nutrients from the soil to the leaves through the transpiration process, where each water molecule released will be replaced by taking water molecules in the soil, the entry of these water molecules allows the entry of nutrients in the soil environment.

The lowest plant height growth was found in sintrong plants (*Crassocephalum crepidioides* (Benth) S.Moore) grown on a mixture of soil and manure with a ratio of 1:2 without shade (P_2N_0). The low plant height without shade can be caused by high light intensity that allows sintrong plants (*Crassocephalum crepidioides* (Benth) S.Moore) which adaptively includes shade plants to experience stress conditions if living in direct exposure to sunlight, high sunlight intensity can reduce the presence of microbes around the root environment where microbes are generally more abundant in humid conditions. Soil media mixed with more manure does not make sintrong plants (*Crassocephalum crepidioides* (Benth. S, Moore) use it for the growth and development of plant height. The excess manure in this study for conditions without shading looks like the texture tends to be harder and not loose). this will interfere with the physical and biological properties of the soil so that the absorption of

Table 1. Average Plant Height Growth

No.	Media + Shade	Mean Plant Height (cm) MST (week after planting)					
		2 MST		6MST		10MST	
		Test Results	Standard Deviation	Test Results	Standard Deviation	Test Results	Standard Deviation
1	P_0N_0	7.33^a	0.58^a	11.00^a	2.00^a	16.67^{ab}	3.79^{ab}
2	P_0N_1	7.67^a	0.58^a	10.00^a	1.00^a	18.33^{ab}	1.53^{ab}
3	P_0N_2	7.00^a	0.00^a	18.67^{bc}	2.08^{bc}	18.33^{bc}	1.53^{bc}
4	P_1N_0	7.33^a	0.58^a	13.33^{ab}	1.53^{ab}	32.33^c	6.81^c
5	P_1N_1	7.67^a	0.58^a	23.00^c	2.65^c	55.67^e	3.51^e
6	P_1N_2	7.33^a	0.58^a	23.00^c	3.46^c	39.00^{cd}	9.54^{cd}
7	P_2N_0	7.33^a	0.58^a	8.67^a	0.58^a	9.67^a	0.58^a
8	P_2N_1	7.33^a	0.58^a	20.00^c	1.73^c	47.33^{de}	5.86^{de}
9	P_2N_2	7.33^a	0.58^a	20.00^c	8.19^c	37.00^{cd}	12.53^{cd}

Notes: Numbers followed by the same letter are not statistically different at the 95% confidence level.
N_0: Shadowless, N_1: 25% Shade, N_2: 50% Shade, P_0: Soil without fertilizer, P_1: Soil: manure (1:1), P_2: Soil : manure (1:2)

nutrients contained in manure does not run optimally which affects the growth and development of sintrong plants (*Crassocephalium crepidiodes* (Benth.S, Moore). Comparison of the growth of sintrong plant height (*Crassocephalium crepidiodes* (Benth. S, Moore) can be seen in the following histogram image.

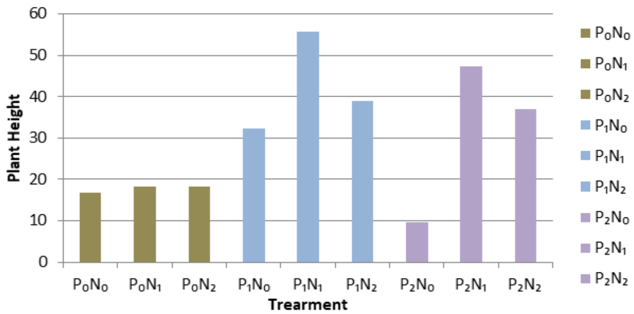


Figure 1. Plant height based on treatment

Figure 1 above shows the response of plant height growth on different planting media and shade shows different results. The provision of balanced manure in this study, a mixture of soil and manure 1: 1 is seen in each treatment of shade and no shade that can help sintrong plants (*Crassocephalium crepidiodes* (Benth.) S. Moore) still maximize the growth process in the difference in sunlight intensity.

Antibacterial: The results of the antibacterial activity test showed that there was a significant effect of sintrong plant extract grown on different planting media and shade treatments on antibacterial values. Antibacterial activity can be seen from the clear zone formed, which means the ability to inhibit the growth of test bacteria such as *Staaphylococcus aereus* and *Eschericia coli* bacteria. The size of the inhibition zone was measured using a caliper. The following is the diameter of the inhibition zone of Sintrong plant extract grown on different planting media and shade.

Table 2. Diameter of inhibition zone for Sintrong plant extract grown

No.	Media + Shade	Diameter of Zone of Inhibition Mean ± Standard Deviation		
		Well diameter	***E.coli***	**S.aureus**
1	P_0N_0	7	14.5 ± 0.0^g	12.6 ± 0.0^f
2	P_0N_1	7	14.1 ± 0.1^f	11.9 ± 0.0^e
3	P_0N_2	7	16.6 ± 0.1^g	13.2 ± 0.2^g
4	P_1N_0	7	11.8 ± 0.0^d	10.0 ± 0.3^c
5	P_1N_1	7	13.7 ± 0.0^e	10.6 ± 0.5^d
6	P_1N_2	7	9.7 ± 0.1^b	9.4 ± 0.0^b
7	P_2N_0	7	8.0 ± 0.0^a	8.0 ± 0.0^a
8	P_2N_1	7	10.2 ± 0.4^c	9.6 ± 0.3^b
9	P_2N_2	7	9.9 ± 0.1^b	9.3 ± 0.1^b

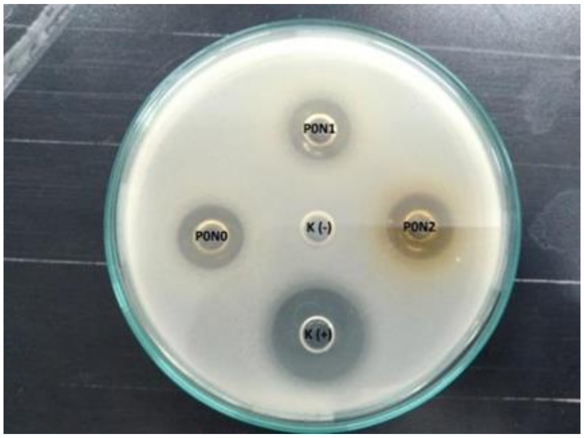
Notes: Numbers followed by the same letter are not statistically different at the 95% confidence level.

N_0: Shadowless; N_1: 25% Shade; N_2: 50% Shade; P_0: Soil without fertilizer; P_1: Soil: manure (1:1); P_2: Soil: manure (1:2)

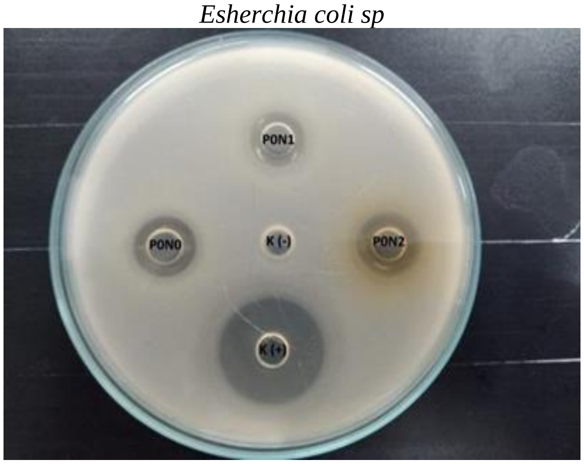
Based on the results of the ANOVA statistical test using the F test through SPPS.23 software, it was found that the *E.coli* antibacterial test showed an F value of 1043.525 with a Sig value. 0.000 (<0.05). The S.aureus antibacterial test showed an F value of 206.855 with Sig. 0.000 (<0.05), then these two bacterial tests show the same conclusion that HO is rejected, which means that the average of *E.coli* and S.aureus bacterial tests for each treatment is significantly different so that Duncan's further test is carried out to see the comparison between each treatment. Duncan's further test results showed that the average diameter of the inhibition zone for the *E.coli* bacteria test for each treatment was divided into 7 (seven) different groups. In addition, the average inhibition zone for *S.aureus* bacteria test for each treatment is also divided into 7 (seven) different groups.

The best antibacterial activity effect was seen in the extract of sintrong plant (*Crassocephalium crepidiodes* (Benth.) S. Moore) obtained from planting without manure at 50% shade (P_0N_2), with a strong inhibitory effect on Eschericia coli bacteria on average. The diameter of the inhibition zone at P_0N_2 was 16.6 mm. Meanwhile, inhibition against Staphylococcus aureus test bacteria was also seen in sintrong plant extract (*Crassocephalium crepidiodes* (Benth.) S. Moore) obtained from planting without manure at 50% shade (P_0N_2) with an inhibition zone diameter of 13.2 mm which is included in the strong category. Sweetman et al (2009) stated that if the diameter of the inhibition zone is less than 5 mm then the inhibitory activity is categorized as weak, the diameter of the inhibition zone 6-10 is categorized as moderate, 11-20 mm is interpreted as strong, 6-10 is categorized as moderate, 11-20 mm is interpreted as strong, with a diameter of 21 mm or more then the inhibitory activity is categorized as very strong. The diameter of the inhibition zone formed can be seen in Fig.2.

Antibacterial activity can work in several ways to damage the bacterial cell wall, change cell permeability, change protein molecules and nucleic acids (Zhang *et al.*, 2023) Inhibit the work of enzymes and inhibit the synthesis of nucleic acids and proteins (Egorov *et al.*, 2018). Antibacterial activity in sintrong plants (*Crassocephalium crepidiodes* (Benth.) S. Moore) in this study was seen both on gram-positive bacteria Staphylococcus aeureus sp and gram-negative bacteria *Escherchia coli* sp. crepidiodes Benth.S.Moore) obtained from the treatment of planting without fertilizer in 50% shade (P_0N_2) plants will synthesize many compounds that can be used to survive conditions of limited nutrients and lower sunlight intensity with a value of 2082 lux. The results of the secondary metabolite profile test LC HRMS treatment P_0N_2 obtained a large compound compared to other treatments, appearing several compounds that have antibacterial functions including Umbeliferone, Cegaba, Xanthine Citral and Eugenol.



Esherchia coli sp

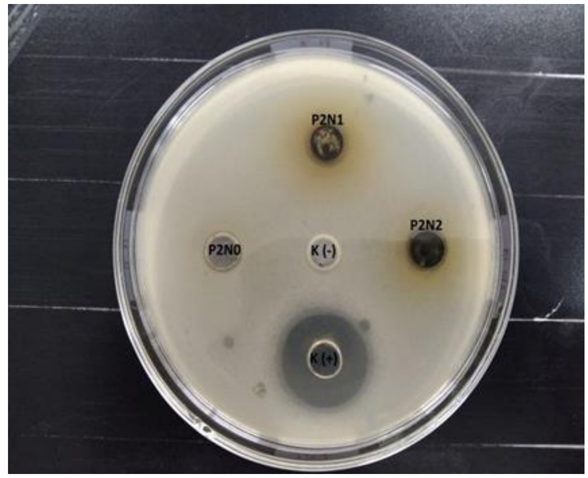


Staphylococcus aureus sp

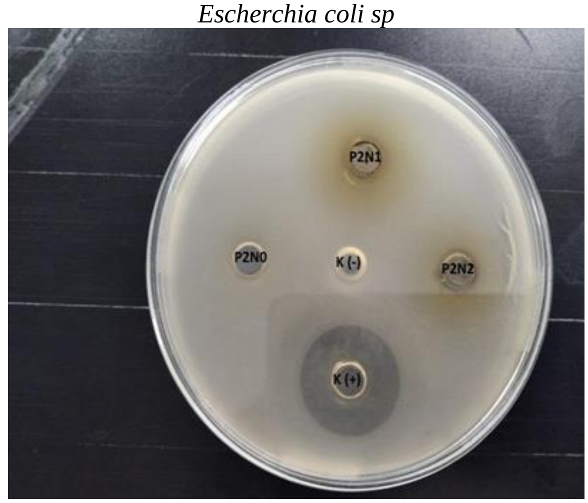
Figure 2. Highest Zone of Inhibition against *Escherchia coli sp* and *Staphylococcus aureus sp* Bacteria

Manure treatment can increase the presence of nitrogen fixing bacteria including Azotobacter sp. which is a heterotrophic Gram negative bacteria. Its optimal growth is 20-30^{oo} C with a pH of around 7.0-7.5. Azotobacter sp. can be found in neutral to alkaline soils, in aquatic environments and in plant rhizospheres. These bacteria are able to grow on various types of carbohydrates, alcohols, and organic acids (Handayanto & Hairiah, 2009). Azotobacter sp has the ability to fix nitrogen, Azotobacter. sp is also able to produce plant growth hormones and antimicrobials (Purba *et al.*, 2021).

The lowest antibacterial activity effect of sintrong plant extract (*Crassocephalium crepidiodes* (Benth.) S. Moore) was seen in planting a mixture of soil and manure in a ratio of 1:2 under shadeless conditions (P_2N_0) with an average inhibition zone value of 8.0 mm against *E.coli* test bacteria and an average inhibition zone of 8.0 mm against S. coli test bacteria. If the diameter of the inhibition zone is less than 5 mm, the inhibitory activity is categorized as weak, the diameter of the inhibition zone 6-10 is categorized as moderate, 11 to 20 mm is interpreted as strong, the diameter of 21 mm or more, the inhibitory activity is categorized as very strong. If the diameter of the inhibition zone is less than 5 mm, the inhibitory activity is categorized as weak, the diameter of the inhibition zone is 6-10 is categorized as moderate, 11 to 20 mm is defined as strong, the diameter of 21 mm or more, the inhibitory activity is categorized as very strong. The following is a picture of the inhibition zone for both test bacteria.



Escherchia coli sp



Staphylococcus aureus sp

Figure 3. The lowest zone of inhibition on *Escherchia coli sp* and Staphylococcus *aureus sp*

Manure treatment can increase the presence of nitrogen fixing bacteria including Azotobacter sp. which is a heterotrophic Gram negative bacteria. Its optimal growth is 20-30^{oo} C with a pH of around 7.0-7.5. Azotobacter sp. can be found in neutral to alkaline soils, in aquatic environments and in plant rhizospheres. These bacteria are able to grow on various types of carbohydrates, alcohols, and organic acids (Handayanto & Hairiah, 2009). Azotobacter sp has the ability to fix nitrogen,

Azotobacter. sp is also able to produce plant growth hormones and antimicrobials Purba *et al.*, 2021).

The results of the correlation test for each treatment of sintrong plants grown in different growing media and shade conditions generally show a strong correlation to antibacterial activity of both Escherchia coli Sp and Staphylococcus aureus sp bacteria with a correlation value of -1 in Escherchia coli Sp bacteria and 0.890 Staphylococcus aureus sp. The results of the LC HRMS test contained secondary metabolite compounds that have functions as antibacterials, namely Muramic, Coumarin, tetrallin, Sarmentosin, and Citral. Where these compounds have different functions in healing diseases. Empirically, sintrong plants have been used as vegetables and are believed to overcome stomach pain, reduce high blood pressure and facilitate menstruation.

***Conclusion*:**The growth response of sintrong plants (*Crassocephalium crepidiodes* (Benth) S. Moore) on different planting media and shade showed different responses for each treatment. The highest growth was obtained in the treatment of sintrong plants grown on a mixture of soil and manure 1: 1 in 25% shade (P_1N_1) with a value of 55.67 cm, while the lowest growth was in the treatment of soil and manure mixture media 1: 2 without shade (P_2N_0) with a value of 9.67 cm. The value of antibacterial activity can be seen from the largest inhibition zone on sintrong plant extract obtained from P_0N_2 media with a strong category, namely 16.6 ± 0.1 mm for inhibition of Escherchia coli sp bacteria and 13.2 ± 0.2 mm for Staphylococcus aureus bacteria. The lowest zone of inhibition of sintrong plant extract obtained from the planting medium of a mixture of soil and manure 1: 2 without shading (P_2N_0) and 8.0 ± 0.0 in the bacteria Staphylococcus aureus sp.

***Author contribution statement*:** N. Malik, Responsible for the overall research; responsible for writing the proposal, producing the research report and writing the article; Muhidin, Muhidin prepared the draft; A. Fristiohady reviewed and finalized the draft.

***Conflict of interest*:** The authors declare no conflict of interest

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***Ethical statement*:** This article contains no experimental measures on humans or animals

***Availability of data and materials*:** We declare that the submitted manuscript is our work, which has not been published before and is not being considered for publication elsewhere.

***Availability Code*:** paper Not applicable

***Consent to participate*:** All authors participated in this research study

***Consent for publication*:** All authors gave their consent to publish this research, article in the journal Journal of Global Innovations in Agricultural Sciences (JGIAS).

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